REMARKS

Claims 1, 2, 4-9, 13-27, 29-43, 45, and 46-49 were pending prior to this response. By the present communication, no new claims have been added, claim 49 has been cancelled and claims 17, 39, 46 and 47 have been amended to define the invention with greater particularity. Accordingly, claims 17, 19, 24, 25, 29, 30-32, 34, 39-43, and 45-48 are currently pending.

The amendments add no new matter, being fully supported by the Specification and original claims. In particular, the amendment to claims 17, 39 and 47 has been made to further clarify the metes and bounds of the claims by requiring that bone marrow early attaching cells are not obtained from the peripheral blood circulation, but are obtained by culturing of bone marrow itself, which is the crux of the invention. Support for the amendment is found throughout the Specification, for example at paragraph [0033], lines 1-5; paragraph [0044], line 1, and paragraph [0100].

The amendment to claim 46 is made to adjust the claim language to that more suitable for a method claim by specifying the processes involved in preparing the composition from aspirated bone marrow.

The Rejection under 35 U.S.C. § 103(a)

A. Applicants respectfully traverse the rejection of claims 17, 19, 24, 25, 35, 39-42 and 45-48 under 35 U.S.C. § 103(a) as allegedly being unpatentable over Kalka et al. (Angiogenesis and Vasculogenesis, Heart, Urban and Vogel, Vol. 25 No. 6 (2002) pages 611-622 (hereinafter "Kalka et al.") in combination with Chiu, U. S. printed publication 2002/0197240 (hereinafter "Chiu"), claim 49 having been cancelled and claims 17 and 48 having been amended. Applicants disagree with the Examiner's assertion that the subject matter of claim 17, as presented, would be obvious to those of skill in the art under 35 U.S.C. § 103(a) over Kalka et al. in view of the disclosure of Chiu. Applicants submit that the subject matter of claim 17 distinguishes over the combined disclosures of Kalka et al. and Chiu by reciting:

"A method for enhancing collateral blood vessel formation in heart or limb muscle tissue, said method comprising:

directly injecting into a site of impaired blood flow in heart or limb muscle tissue an effective amount of early attaching cells obtained from autologous bone marrow, which early attaching cells have been transfected in vitro with an adenoviral vector comprising a polynucleotide encoding one or more angiogenic factors selected from hypoxia inducing factor-1 (HIF-1), endothelial PAS domain protein 1 (EPAS1), Monocyte Chemoattractant Protein 1 (MCP-1), granulocyte-monocyte colony stimulatory factor (GM-CSF), PR39, a fibroblast growth factor (FGF), and a nitric oxide synthase (NOS)."

Applicants respectfully submit that Kalka et al. neither teaches nor suggests the use of early attaching cells obtained by culturing bone marrow as gene therapy expression cells for delivering to ischemic muscle tissue one or more angiogenic agents that enhance development of collateral blood supply, wherein the angiogenic agent(s) are expressed by the early attaching cells in vitro. Thus, the combined teaching of Kalka et al. and Chiu fail to disclose or suggest the claimed invention under 35 U.S.C. § 102 or 103(a). Applicants are the first to invent a method whereby the step of extracting EPCs from peripheral blood can be eliminated and replaced by culturing bone marrow cells to obtain therefrom early attaching cells for injection into ischemic muscle tissue in heart or limb to induce development of collateral blood vessels. Moreover, Applicants are the first to disclose that cells obtained from bone marrow by culturing of such cells can be transfected by and will express in vitro DNA encoding a therapeutic angiogenesis factor to produce conditioned medium containing therapeutic angiogenesis factors.

The Examiner asserts that those of skill in the art would be motivated to improve upon Kalka's disclosed methods because of the known paucity of EPCs in peripheral circulation. However, the Examiner fails to document that the initial population of EPCs in the early attaching cells obtained by culturing bone marrow was known by those of skill in the art to be substantially greater than that can be extracted from peripheral blood circulation. Moreover, Applicants submit that it was not know in the art, nor does the Examiner provide evidence showing, that bone marrow cells extracted from peripheral circulation are substantiallty "the same" as those extracted from peripheral blood circulation

or would suggest use of those obtained by culturing bone marrow. In fact, Applicants submit that it was well known in the art that PECs, such as those disclosed by Kalka, are influenced in their development by the in vivo environment in which they are found (See for example, Chiu of record herein). In addition, the bone marrow cells used in the experiments described by Kalka et al. are not identified as autologous to the patient treated. Therefore, Applicants respectfully submit that the Examiner fails to show that the bone marrow cells used by Kalka et al. are the same or would function the same in vivo as the early attaching cells obtained by culturing bone marrow.

In addition, Applicants respectfully submit that Kalka et al. fail to suggest that bone marrow cells obtained from bone marrow aspirate of the subject to be treated can be cultured in vitro to obtain an expanded population of early attaching cells compatible with the subject so that no immune reaction will result from injection of such cells. Kalka et al. are completely silent regarding use of autologous cells. In fact, Applicants submit that Kalka et al. "teach away" from expansion of cells in vitro by recommending expansion of the population of circulating EPCs *in vivo* by prior injection of VEGF (or free plasmid DNA encoding a therapeutic angiogenic factor) into peripheral blood of the donor. Similarly, Applicants respectfully submit that Kalka et al. fail to suggest that bone marrow cells however obtained can be successfully transfected (i.e, at a suitable transfection ratio) by one or more adenoviral vectors encoding the angiogenic factors and will express such angiogenic factors as are specified in claim 17, either in vitro or in vivo.

In addition, Applicants submit that Kalka et al. are absolutely silent regarding "further culturing" of such transfected early attaching cells so as to express into conditioned medium one or more of the said therapeutic angiogenic factors. Thus, Kalka et al. are silent regarding a method for enhancing collateral blood vessel formation in heart or limb muscle tissue by injecting into such tissue a composition that will contain expressed transgenic angiogenic factors (as well as the transfected early attaching cells, which studies have shown will continue to express the transgenic factors when the composition has been injected into the subject). Much less does Kalka et al. suggest the invention method as recited by claim 47 in which bone marrow cells are used as expression cells for producing in vitro a transgenic stimulatory angiogenic factor to "jump start" in the cells the cascade of

endogenous angiogenic factors that blood marrow-derived cells were known to produce when implanted in ischemic tissue.

In addition, with regard to "therapeutic angiogenesis", Applicants submit that Kalka et al. fail to enable a method involving injection of transfected bone marrow cells of any provenance, because Kalka et al. describe only expression of a marker protein by transfected EPCs. Moreover, although Kalka et al. may suggest the possibility of "postnatal neovascularization" in adults, Kalka's suggestion, if any such be, is accompanied by warnings that stimulating postnatal neovascularization may also "stimulate a pathological neovascularization" (Kalka et al., page 27). "Further research must ... be carried out ...with regard to a possible therapeutic use of endothelial progenitor cells within the scope of a cell therapy for the regeneration of ischemic tissue" (Kalka et al., page 28). In view of these warnings, Applicants submit that the Kalka reference would prohibit a well-founded belief in the success of such a method, if those of skill in the art were motivated "to try" administration of recombinant EPCs of any type for adult angiogenesis for therapeutic purposes.

Thus, even if those of skill in the art were motivated by the disclosure of Kalka et al. "to try" substitution of transgenic cells obtained according to the claimed methods for use in "therapeutic angiogenesis", Applicants submit that Kalka's statements fail to constitute obviousness under the statute because the requisite expectation of success is missing and, indeed, warned against.

Furthermore, with respect to the invention of claim 46, there is no evidence provided in the cited art or knowledge of the art as referenced by the Examiner that cells obtained from cultured bone marrow aspirate, if transfected with one or more adenoviral vector(s) encoding a stimulatory angiogenic factor, could be used to prepare conditioned medium containing transgenically expressed angiogenic factors for use as an adjunct to angiogenic cell therapy. Applicants submit that the reference discloses only "the possibility of utilizing" transfected EPCs (obtained from peripheral circulation) for formation of vessels by over-expression of VEGF (Kalka et al, page 27). Actual transfection of EPCs disclosed in Kalka et al. pertains to expression of β-galactosidase in cells of the endothelium cell line in the bone marrow of a mouse (Kalka et al., pp. 18-19). Again, Applicants submit that Kalka et al, at best, would motivate those of skill in the art only to "try" injection into

ischemic tissue of transfected EPCs obtained from peripheral circulation for secretion in vitro or in vivo of a stimulatory angiogenic factor.

The Examiner, in fact, admits that "Kalka et al. do not teach a method for enhancing collateral blood vessel formation by using early attaching cells obtained from bone marrow transfected with an adenoviral vector encoding one or more of the angiogenic factors such as HIF-1, EPAS1, MCP-1 GM-CSF, etc. (Office Action, page 3). To overcome the differences between Kalka et al. and the claimed subject matter, the Examiner relies upon Chiu. However, disclosure of Chiu pertains primarily to myogenesis, not to angiogenesis. Chiu discloses injection into an MI patient of autologous marrow stromal cells that have been modified to express a cardiomyocyte phenotype in vitro. Thus, the therapeutic goal of Chiu is myogenesis, not angiogenesis as in the claimed methods. A secondary group of marrow stromal cells Chiu specifically describes as being "non-modified" are said to "differentiate into angiogenesis" to provide blood flow for development of the implanted cardiomyocyte-producing cells (Chiu [0160] and [0161]). Thus, Chiu fail to suggest transfection of angiogenesis-producing cells.

Moreover, Applicants submit that Chiu is absolutely silent regarding use of transfected cells derived from culture of bone marrow cells for any purpose. Chiu refers to use of "labeled cardiac myocytes and fibers" (Chiu et al, paragraph [0037]), only to track incorporation of myocytes into ischemic or damaged heart muscle, but does not contemplate transfection of MSCs with any therapeutic angiogenic factor, let alone one that will be expressed in vitro for administration to a subject in conditioned medium. Instead, as in the Kalka reference, Chiu stresses that therapeutic use of implanted MSCs is in the experimental stage and to be worked out in the future:

> The role of cytokines and other growth factors will . . . be examined in the future. Further still, methodologies for transplanting autologous MSCs in patients to improve cardiac function will be optimized in future studies.

(Chiu, paragraph [0040].

Despite the above-described differences between the claimed subject matter and the cited art as well the reservations and uncertainties disclosed by both pieces of art cited, the Examiner asserts that their combined disclosures would suggest Applicant's methods for the implantation of transfected (i.e.

"modified) marrow stromal cells to cause development of collateral blood flow in ischemic cardiac and limb tissue.

However, Applicants respectfully submit that the combined references would, at the most, motivate those of skill in the art "to try" the invention as defined by the claims at issue here, but would not provide those of skill in the art with an expectation of success. Consequently, Applicants respectfully submit that the differences between the cited art and the present invention, as recited in claims 17 and 39, are such that the subject matter as a whole would not have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains.

In view of the above remarks, Applicants respectfully request reconsideration and withdrawal of the rejection of claims 17, 19, 24, 25, 34, 39-42 and 45-48 under 35 U.S.C. §103(a) as being unpatentable over the combination of Kalka et al. and Chiu.

B. Applicants respectfully traverse the rejection of claims 29-32 under 35 U.S.C. § 103(a) as allegedly being unpatentable over Kalka et al. and Chiu as applied above and further in view of Hamawy et al. Smith et al. and Li et al. Applicants' remarks above regarding the differences between the disclosures of Kalka and Chiu and the invention methods for enhancing collateral blood vessel formation applies equally to the rejection of claims 29-32 and is incorporated here by reference.

In addition Applicants disagree with the Examiner's reliance upon Hamawy, et al., Smith et al. and Li et al. as providing the suggestion, teaching or motivation needed for those of skill in the art to overcome the differences between the combined disclosures of Kalka et al. and Chiu such that the subject matter of claims 29-32 would have been obvious under 35 U.S.C. § 103(a) to those of skill in the art at the time of the invention.

The Examiner alleges, for example, that Hamawy et al. identify over 20 angiogenic factors associated with revascularization of ischemic muscle tissue, including the VEGF disclosed by Kalka et al. and the (FGF)s as recited in claim 39 (Office Action, page 5). In summary, the Examiner states: "Further, given the level of skill in the art at the time of invention there would

have been a reasonable expectation of success in replacing one angiogenic factor with another" (Office Action, page 6). However, the Examiner fails to point out any passages in Hamawy, et al., Smith et al. and Li et al., other than those disclosing yet another angiogenic factor endogenously produced by an individual suffering from ischemic muscle tissue, that would overcome the differences between the combined disclosures of the primary references (as discussed above and incorporated here) and the subject matter of claims 29-32, which contain all the requirements of claim 17. Accordingly, Applicants submit that the cited art fails to establish prima facie obviousness of the subject matter of claims 29-32 under 35 U.S.C. § 103(a) and reconsideration and withdrawal of the rejection are respectfully requested.

C. Applicants respectfully traverse the rejection of claim 43 under 35 U.S.C. § 103(a) as allegedly being unpatentable over Kalka et al. and Chiu as above and further in view of Tomika. With regard to the primary references cited, Applicants' remarks above regarding the differences between the invention methods for enhancing collateral blood vessel formation and the disclosures of Kalka et al. and Chiu apply equally to the rejection of claim 43, which incorporates all the limitations of claim 17, and are incorporated here by reference.

The Examiner relies upon Tomika as disclosing that when obtaining bone marrow derived cells it is beneficial to have an anticoagulant present and alleges with respect to claim 43: ". . . it would have been obvious to add heparin to the aspirate to obtain the benefit of preventing clotting or coagulation of a composition comprising bone marrow, which in turn comprises cells that are expanded/transfected *ex vivo.* " (Office Action, page 7)

However, the Examiner fails to point out any passages in Tomika that pertain to the present claims, other than those referring to the utility of including an anticoagulant in a composition comprising bone marrow cells. In particular the Examiner fails to point out how Tomika would supplement and overcome the differences between the Kalka/Chiu combination of references (incorporated from above) and the subject matter of claim 43, which depends from and includes all the limitations of claim 17. Accordingly, Applicants submit that the cited art fails to

establish prima facie obviousness of claim 43 under 35 U.S.C. §103 and reconsideration and withdrawal of the rejection are respectfully requested.

The Rejection under 35 U.S.C. § 112, Second Paragraph.

Applicants respectfully traverse the rejection of claims 45 and 46 under 35 U.S.C. § 112, second paragraph for being indefinite as allegedly failing to point out and claim the subject matter of the invention.

With regard to the word "derived" in these claims, the Examiner asserts that the nature and number of derivative processes referred to are unknown so that those of skill in the art would be uncertain of the metes and bounds of the claims. To overcome the grounds of the rejection Applicants have amended claims 45 and 46 to delete the phrase "derived from bone marrow" and to substitute in its place the phrase "obtained by culturing bone marrow". Thus, the amendment clarifies that the early attaching cells are not obtained from the peripheral circulation of a subject, but instead are obtained by culturing of the bone marrow aspirated from a subject.

Accordingly Applicants submit that claims 45 and 46 as currently amended meet all requirements under 35 U.S.C. § 112, second paragraph. Reconsideration and withdrawal of the rejection of claims 45 and 46 as being indefinite, therefore, are respectfully requested.

Respectfully submitted,

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